I have examined of an Orang which had cut its first upper and lower incisors it is $-7°$, in one young Chimpanzee it is $-9°$, and in another older which has cut its second but not its third molar the angle is $-16°$.

Thus it would appear that a sharp distinction is established by this character between the higher apes and existing men at a very early stage of their career.

The sagittal section afforded by the Chimpanzee with a nasion angle of $-16°$ is shown enlarged and superposed on the corresponding section of La Chapelle-aux-Saints in fig. 3. A remarkable correspondence will be observed. The lambdas are not far apart, the bregmas differ by only 1° and the nasion angles are almost identical.

Observations on the Changes seen in Living Cells during Growth and Division.

By T. S. P. STRANGEWAYS, Lecturer in Special Pathology in the University of Cambridge.

(Communicated by Mr. W. B. Hardy, Sec.R.S. Received May 11, 1922.)

(From the Laboratories of the Cambridge Research Hospital.)

[PLATES 2–5.]

The observations recorded in this paper were made on cultures *in vitro* of embryonic and adult chick tissues. The cultures chiefly studied were choroidal cells from the eyes of seven to nine days' chick embryos and cartilage cells from knee-joints of adult fowls.

The method of cultivation was embedding small fragments of tissue on a coverslip in one drop of chick plasma, to which was added one drop of embryo chick extract. The coverslip was inverted over a hollow glass slide, sealed with melted paraffin wax, and at once placed in an incubator at 39° C. The tissues were sub-cultured every second day. The cells observed were found in cultures which had been growing for 24 hours in the incubator after sub-culture.

The changes described were seen equally well in cultures from the embryonic choroid or from adult fowl cartilage, but in order to avoid any possible confusion, the description of the changes seen will be confined to cells from the embryonic choroid.

During observation, the microscope and cultures were kept at a temperature 39° C. in Nuttall's thermostat. The cells were watched over

an Abbe condenser and under a 1/10 oil-immersion objective. The source of illumination was a Welsbach gas mantle. Under these conditions, the cells and the changes occurring in them during growth can be watched for many hours.

The cytoplasm appears as a clear homogeneous jelly-like substance with no obvious cell wall, although its limits can be readily discerned both in cells lying free in the plasma and in those growing on the surface of the coverslip.

The outline of the growing cell is constantly changing (Plates 2–5), and larger or smaller processes of cytoplasm are thrown out and withdrawn as in amœboid movement. This movement is slow, and often is only noticeable on watching the cell for some minutes; at other times the movement is so rapid that it can be actually observed. The rate of movement varies in different cells and in different cultures; and also with the rate of growth of the culture itself. As will be seen from the figures the movement takes place all round the cell, which, however, shows a tendency to assume an elongated shape, and the end which is nearest the periphery of the growth (figs. 40–67) usually shows more active movement. By these movements the cell may slowly change its position either in the plasma or on the surface of the coverslip. Fine rod-like filaments of cytoplasm, projecting from the cells and showing amœboid movement, may also be seen; experience and close observation are required in order to distinguish these filaments from mitochondria. The actual size of the cell appears to vary somewhat from hour to hour.

Fine rod-like structures, which are described as mitochondria, may be seen in many cells. On careful watching, these may be seen moving in the cytoplasm from one part to another. Intra-vital staining by Janus black brings them out more clearly as blue-black rods.

In the cytoplasm a number of minute refractile granules are seen; these constantly change their position, and move freely in the cytoplasm from one part to another—they move past and around the nucleus, but have never been observed to enter this body; these granules vary in number from time to time. On intra-vital staining by polychrome methylene blue, some of these granules are stained blue, others red. On staining by iron hæmatoxylin they are not seen after differentiation.

In some cells clear droplets which stain readily by the usual stains for fat may be distinguished. These vary in size and number but in vigorously growing cells they are not seen.

The nucleus is well defined, usually oval in shape, and can be clearly seen lying in the cytoplasm but sharply differentiated from it; although generally oval in contour it may appear round (fig. 65). When first formed after

mitosis the nucleus is relatively small (fig. 13), but it soon increases in size. The size of the nucleus is, however, not constant in a growing cell, it appears to vary somewhat with the size of the cell itself. The nucleus wanders about the cell freely, being sometimes in the centre (fig. 27), at other times at one or other pole (fig. 35), but with a tendency to lie more or less in the centre of the cytoplasm, its position appears to depend largely on the movements of the cytoplasm. In some cells where large cytoplasmic processes are thrown out the nucleus may move out with these processes, if the process is withdrawn the nucleus draws back with it.

The nucleus contains one or more irregular structures, which stain intensely with iron hæmatoxylin, and will be referred to in the following description as "nucleoli" in order to avoid introducing new names, but it should be noted that this name is used for convenience of description only. These structures require further study in order to determine their composition and function. The nucleoli vary in size, shape and number; and in vigorously growing cells one or two of these are present and they are constantly altering in shape and size. During the growth of the cell a single nucleolus may divide into two nucleoli (fig. 17), and these remain as separate bodies for a time and then unite again (fig. 28). This division and union may occur more than once (figs. 33 and 58). The process of division and union of nucleoli has been watched on many occasions, and has also been confirmed by stained specimens.

There are also seen in many choroidal cells brown pigment rods the number of which varies greatly in different cells. The pigment rods when present in a cell show interesting changes in position. They lie either separately or together, and generally show more or less Brownian movement; but in addition to this, if watched carefully, some of them may be seen to move more or less rapidly and irresponsibly in the cytoplasm, like guinea-pigs in a run. The cause of this movement has not been determined.

In cells which are at rest, or show a lag in growth, or are growing under abnormal stimuli, it is possible to make out other changes—these, however, will not be described in this paper.

The changes preceding mitosis take place in vigorously growing cells rapidly. The processes of cytoplasm retract (fig. 72) and the cell assumes an oval or rounded shape (fig. 73)—the nucleoli become fainter (fig. 72) and then appear as hazy granules (fig. 73). This change takes from 2–10 minutes under favourable conditions. Then the prophase is seen (fig. 74) as a number of fine threads lying within the nucleus. These are frequently seen in active writhing movement like eels in a box. The outline of the nucleus at this stage suddenly disappears, and the chromosomes rapidly arrange themselves at right angles to the spindle which with the centrosomes can now be seen (figs. 2

and 76). The spindle has not been observed preformed outside the nucleus nor is it seen until the nuclear outline has disappeared. The time between the prophase stage and the appearance of the spindle varies somewhat, but on the average is about 8 minutes. Within 5 to 10 minutes after the spindle is seen the chromosomes begin to draw apart towards the centrosomes, and show clearly as finger-like processes, being drawn towards either pole of the cell (figs. 3, 4, 5, 6, 7, and 77, 78) which has by this time taken on an oval shape.

At this stage most interesting changes at the outline of the cell develop. Small balloons of cytoplasm project from the surface of the cell (figs. 6, 7, 8, 9, 10), these remain for a few seconds and then collapse. The granules in the cytoplasm can be seen (figs. 6 to 10), flowing in when the balloons are formed and streaming out when they collapse. This movement continues for about 6 minutes, new balloons being formed as the others collapse. This balloon formation is unlike amœboid movement, and appears due to local changes of surface tension. During this stage the cell begins to divide and the outline becomes irregular; two masses of cytoplasm form (figs. 8, 9, 10), which are constantly altering in shape; these gradually pull apart until joined only by fine filaments of cytoplasm and a few mitotic threads (fig. 11).

The granules in the cytoplasm do not appear to take any active part in mitosis.

During this time the chromosomes, which, as stated above, when first drawn apart show finger-like processes (fig. 4), seem to fold in upon themselves and gradually form a small faint irregular body (figs. 8, 9, 10, 11). Within a few minutes after the two cells are formed, this body is surrounded by a clear zone which is clearly differentiated from the cytoplasm, and a small nucleus, with a nucleolus lying within it, appears in both cells; these nuclei are clearly seen about 28 minutes after the chromosomes draw apart. The impression given by many observations is that the formation of the nucleolus is intimately associated with the disappearance of the chromosomes; but further observation and study are required on this point. During the process of division the granules have been in a state of active movement, flowing from one side or other of the two cytoplasmic masses (figs. 1 to 10). Before division finally takes place they become distributed in fairly even numbers between the two newly-formed cells. After division the irregular movement of the cytoplasm gradually diminishes, and the two cells assume within 1 or 2 hours the characteristic shape and size of mature growing cells and show nuclei of normal size. The time from the beginning of one division to the beginning of the next is between 11 to 12 hours.

The above observations show clearly that the cell and the structures seen within it are never at rest during growth and division.

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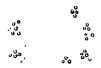

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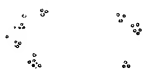

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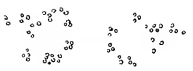

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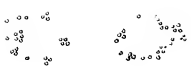

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observation discontinued

11 a.m.

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observation continued

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nucleolus divides
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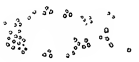

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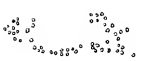

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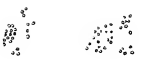

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1 p.m.

nucleoli join
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1.10

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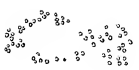

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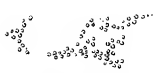

nucleolus divides
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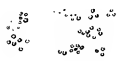

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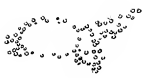

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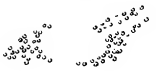

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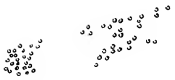

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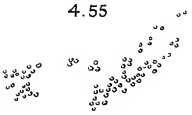

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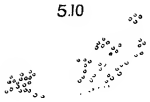

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57

nucleoli join

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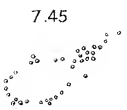

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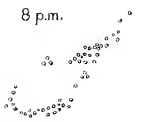

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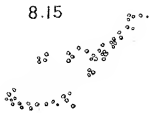

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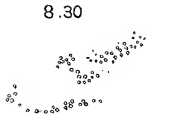

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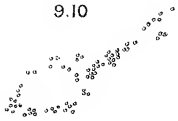

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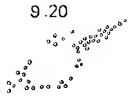

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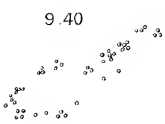

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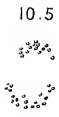

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A series of observations on the time occupied by various stages of mitosis in vigorously growing cells gave the following results:—

(1) From the beginning of the prophase (that is, from the commencement of the breaking up of the nucleolus) to the spindle being clearly seen, the time varied, in thirty cells, from 3 to 20 minutes, the average being 7 minutes.

(2) From the formation of the spindle to the division of the cytoplasm into two cells the time varied, in fifty-five cells, from 12 to 47 minutes, the average being 20 minutes.

(3) From the formation of the spindle to the appearance of two daughter nuclei, the time varied, in sixty cells, from 11 to 50 minutes, the average being 28 minutes.

(4) The period during which the cell outline showed "balloon" formation varied, in fifty-five cells, from 1 to 16 minutes, the average being 6 minutes.

(5) The time occupied for complete division of a cell, from the beginning of the prophase to the daughter nuclei being clearly seen, varied, in thirteen cells, from 23 to 65 minutes, the average being 34 minutes.

It should be noted that in cells, which for any reason show a lag in growth, any or all of the stages of mitosis may be considerably prolonged.

Owing to the difficulty of determining the commencement and termination of prophase, metaphase, anaphase, and telophase, the periods occupied by these phases have not been recorded, but Lewis (1917)* has discussed their probable duration in an article on the duration of the various phases of mitosis, in which he also discusses the observations recorded by Lambert (1913)† and Levi (1916).‡

Both Lewis and Levi worked with chick embryo cultures, and it is interesting to note that, although Lewis used Locke's solution as a culture medium and Levi chick plasma, the results of both these observers agree closely with those recorded above, in which chick plasma and embryonic chick extract were used as the medium for cultivation.

I have been unable to find any record by other observers of the period occupied and the changes seen in cells watched from one division to the next when cultivated *in vitro*.

The expenses in connection with this study were met by a grant from the Medical Research Council.

* Lewis, W. H., and Lewis, M. R., 'Anat. Record,' vol. 13, p. 359 (1917).

† Lambert, R. A., and Hanes, F. M., 'Virchow's Arch.,' vol. 211, p. 89 (1913); Lambert, R. A., 'Journ. Exper. Med.,' vol. 17, p. 499 (1913).

‡ Levi, G., 'Arch. Ital. Anat. Embriol.,' vol. 15, p. 243 (1916).

10am

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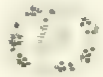

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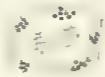

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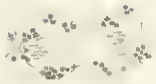

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observation discontinued

11am

16

observation continued

11 10

nucleolus divides
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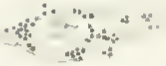

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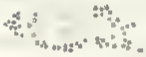

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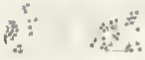

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1 p m

nucleoli join
28

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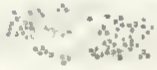

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nucleolus divides
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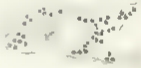

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2 40

37

2 50

38

3 p.m

39

3 15

40

3 30

41

3 40

42

3 50

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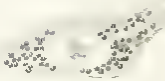
4 p.m



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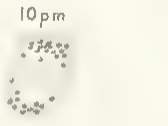

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